

provides recombinant expression cassettes, host cells, transgenic plants, and antibody compositions. The present invention provides methods and compositions relating to increasing essential amino acid content of plants for feed.

6. Please replace Figures 1 and 2 in their entirety with amended Figures 1 and 2 provided herewith as **Appendix A**. Additions to Figures 1 and 2 are shown in red ink. No deletions were made.

7. Please replace the sequence listing in its entirety with the revised sequence listing attached as **Appendix B**. A disk with the revised sequence listing shown in **Appendix B** in computer readable format is also enclosed.

To the claims:

Please delete claims 1-8, 33-47, 49-53 and 88-95.

Please amend the claims as follows:

9. (three times amended) A polypeptide with at least 30% sequence identity to the polypeptide of Seq. ID No. 2 and comprising greater than fifty amino acids in length and modified in order to have a composition selected from one of the following: at least 15-35 mole % lysine, at least 5-15 mole % methionine, at least 6-25 mole % threonine, and at least 4-9 mole % tryptophan; wherein the % sequence identity is based on the entire sequence and is determined by BLAST 2.0 using default parameters.

10. (twice amended) A polypeptide comprising Seq. ID No. 2 or 4 modified to contain seven or more non-native essential amino acid residues at positions corresponding to the positions in Seq. ID No. 2 selected from 1, 8, 11, 17, 18, 19, 20, 22, 23, 31, 34, 38, 40, 41, 47, 49, 56, 58, 59, 60, 61, 62, 63, 65, 67, 69, 73, 75, 76, 78, 79, 81, 82 or combinations thereof; and further provided

that the polypeptide has at least 30% sequence identity to the polypeptide of Seq. ID No. 2 or 4, wherein the percent identity is determined by Blast 2.0 using default parameters.

11. (twice amended) The polypeptide of Claim 10 wherein the essential amino acid residues comprise isoleucine, lysine, threonine, tryptophan, methionine, leucine, valine or combinations thereof.

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12. (once amended) The polypeptide of Claim 10 wherein the protein exhibits reduced inhibitory activity against chymotrypsin, subtilisin or elastase when compared with the inhibitory activity against chymotrypsin, subtilisin or elastase exhibited by wild type CI-2.

13. (once amended) The polypeptide of claim 10 wherein the polypeptide further comprises one or more of the following modifications: V32T; E45T; D64T; D74T; or A77T.

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14. (once amended) The polypeptide of claim 10 comprising one of the following pairs of substitutions: T22C and V82C; or E23C and R81C.

15. (no change) The polypeptide of Claim 10 further comprising an amino-terminal extension.

16. (once amended) The polypeptide according to claim 15 wherein the amino terminal extension comprises essential amino acids.

17. (twice amended) The polypeptide of Claim 15 wherein the amino-terminal extension is a methionine or a signal peptide.

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18. (twice amended) The polypeptide of Claim 15 wherein the amino-terminal extension comprises at least one to about eighteen additional residues corresponding to amino acid residues 1 to 18 of Seq. ID No. 2 or 12.

19. (twice amended) A polypeptide comprising two or more of the following modifications corresponding to positions in Seq. ID No. 2 selected from the group consisting of:

H18A, H18I, H18L, H18V, H18M, N19K, N19T, L20M, L20I, L20V, E23T, E23K, S31T, S32K, E34K, E34T, V38M, V38I, V38L, L40M, L40I, L40V, Q41K, Q41T, Q47K, Q47T, I49M, I49I, I49L, I49V, I56K, I56T, M59G, R62K, R62T, I63M, I63L, I63V, R65K, R65T, R67K, R67T, F69W, L73K, L73T, N75K, N75T, Q78K, Q78T, V79T, V79K, R81K, and R81T; and

further provided that the polypeptide is a nutritional supplement and has at least 30% sequence identity to the polypeptide of Seq. ID No. 2, wherein the percent identity is determined by Blast 2.0 using default parameters.

20. (once amended) The polypeptide according to claim 19 wherein the modifications further comprise one or more of the following pairs of substitutions: E23C and R81C; T22C and V82C; or V53C and V70C.

21. (twice amended) A polypeptide comprising two or more of the following modifications corresponding to positions in Seq. ID No. 2 selected from the group consisting of:

H18A, H18M, N19K, L20M, T22C, E23T, E23C, S31T, E34K, V38M, L40M, Q41K, Q47K, I49M, I56K, M59G, R62K, I63M, R65K, R67K, F69W, L73K, N75K, Q78K, V79T, R81K, R81C, and V82C; and

further provided that the polypeptide is a nutritional supplement and has at least 30% sequence identity to the polypeptide of Seq. ID No. 2, wherein the percent identity is determined by Blast 2.0 using default parameters.

22. (twice amended) The polypeptide of claim 21, further comprising substituting a tryptophan at position 61.

23. (no change) The polypeptide of claim 22, further comprising threonine at one or more of positions 32, 45, 53, 64 or 70.

24. (once amended) The polypeptide of claim 22 wherein the modifications comprise one or more of the following pairs of substitutions: E23C and R81C; or T22C and V82C.
25. (no change) The polypeptide according to claim 22 further comprising an insert in the active site loop region that is enriched in essential amino acids for the purpose of nutritional enhancement.
28. (twice amended) A polypeptide comprising three or more non-native essential amino acids replacing native amino acids at positions corresponding to Seq. ID No. 2 and selected from the group consisting of positions 1, 8, 11, 17, 18, 19, 20, 22, 23, 31, 32, 34, 38, 40, 41, 45, 47, 49, 56, 58, 59, 60, 61, 62, 63, 64, 65, 67, 69, 73, 74, 75, 76, 77, 78, 79, 81 and 82; and excluding V and W at position 56; K, V and W at position 58; W, V and K at position 59; T, I and K at position 60; V and W at position 61 and V and F at position 62; and further provided that the polypeptide is a nutritional supplement and has at least 30% sequence identity to the polypeptide of Seq. ID No. 2, wherein the percent identity is determined by Blast 2.0 using default parameters.
29. (twice amended) A polypeptide comprising Seq. ID No. 6, 8, 10, 12, 14, 16, 18, 20 or conservatively substituted variants thereof.
30. (twice amended) A polypeptide comprising at least twenty three contiguous amino acids of Seq. ID Nos. 6, 8, 10, 12, 14, 16, 18 or 20.
31. (twice amended) A polypeptide with more than 79% sequence identity to the polypeptide of Seq. ID No. 20, wherein the percent sequence identity is determined by GAP analysis using Gap Weight of 12 and Length Weight of 4.
32. (once amended) A polypeptide comprising a conservatively substituted variant of the polypeptide of Seq. ID No. 20 that is immunologically reactive

with antibodies against the polypeptide of Seq. ID No. 20 and not the polypeptide of Seq. ID No. 2.

54.(twice amended) The polypeptide of claim 9, wherein the polypeptide is modified in order to have a composition of at least 5-15 mole % methionine.

55.(twice amended) The polypeptide of claim 9, wherein the polypeptide is modified in order to have a composition of at least 6-25 mole % threonine.

56.(once amended) The polypeptide of claim 10 wherein the non-native essential amino acid residues comprise lysine and the positions correspond to the positions in Seq. ID No. 2 selected from 1, 8, 11, 17, 19, 34, 41, 56, 59, 62, 65, 67, 73 or combinations thereof.

57.(no change) Food or feed comprising the polypeptide of claim 30.

58.(no change) Food or feed comprising the polypeptide of claim 31.

59.(twice amended) A polypeptide with at least 60% sequence identity to the polypeptide of Seq. ID No. 2 comprising greater than fifty amino acids in length and comprising more than seven lysine amino acid residues.

60.(twice amended) The polypeptide of Claim 59, further comprising more than ten lysine amino acid residues.

61.(once amended) A polypeptide selected from the group consisting of:

- (a) a polypeptide comprising Seq. ID Nos. 6, 8, 10, 12, 14, 16, 18 or 20; and
- (b) a polypeptide comprising any one of Seq. ID Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20, wherein said polypeptide has been modified to contain more than four non-native essential amino acids at positions corresponding to Seq. ID No. 2

Seq. ID No. 4
positions 19-53 and 70-83, and wherein said modified polypeptide has at least 30% sequence identity to the polypeptide of Seq. ID No. 4 or 20, the percent sequence identity determined by Blast 2.0 using default parameters.

62. (once amended) A polypeptide comprising a conservatively substituted variant of the polypeptide of claim 61, with the proviso that the polypeptide is not Seq. ID No. 2, 4 or 35-53.
63. (no change) The polypeptide of claim 61, wherein the essential amino acid is isoleucine, lysine, tryptophan, methionine, threonine, or mixtures thereof.
64. (no change) The polypeptide of claim 61, wherein the essential amino acid is lysine.
65. (no change) The polypeptide of claim 61, further comprising an amino terminal extension.
66. (once amended) The polypeptide of claim 65, wherein the amino terminal extension comprises a methionine.
67. (no change) The polypeptide of claim 65, wherein the amino terminal extension comprises essential amino acids.
68. (no change) The polypeptide of claim 61, further comprising at least one non-native cysteine.
- Seq. ID No. 2*
69. (once amended) The polypeptide of claim 68, wherein the non-native cysteine is at one or more positions corresponding to Seq. ID No. 2 positions 23, 81, 22, 82, 53 or 70.

70.(no change) The polypeptide of claim 61, further comprising at least two non-native cysteines.

71.(once amended) The polypeptide of claim 70, wherein the non-native cysteines are at one or more of the following pairs of positions: T22C and V82C; or E23C and R81C.

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72.(once amended) A polypeptide comprising any one of Seq. ID Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20, wherein said polypeptide has been modified to contain at least one non-native disulfide bond and more than four non-native essential amino acids in positions corresponding to Seq. ID No. 2 positions 19-83, and wherein said modified polypeptide has at least 30% sequence identity to the polypeptide of Seq. ID No. 4 or 20, the percent sequence identity determined by Blast 2.0 using default parameters.

73.(no change) The polypeptide of claim 72 wherein the non-native essential amino acids are lysine.

74.(once amended) A polypeptide comprising any one of Seq. ID Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20, wherein the polypeptide has been modified to contain more than seven non-native essential amino acids in positions corresponding to Seq. ID No. 2 positions 19-53 and 70-83, and wherein said modified polypeptide has at least 30% sequence identity to the polypeptide of Seq. ID No. 4 or 20, the percent sequence identity determined by Blast 2.0 using default parameters.

75.(no change) The polypeptide of claim 74 wherein the non-native essential amino acids are lysine.

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76.(once amended) A polypeptide comprising any one of Seq. ID Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20, wherein the polypeptide has been modified to contain at least eleven non-native essential amino acids in positions

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corresponding to Seq. ID No. 2 positions 19-83, and wherein said modified polypeptide has at least 30% sequence identity to the polypeptide of Seq. ID No. 4 or 20, the percent sequence identity determined by Blast 2.0 using default parameters.

77. (no change) The polypeptide of claim 76 wherein the non-native essential amino acids are lysine.

78. (once amended) A polypeptide having at least 60% sequence identity to the polypeptide of Seq. ID No. 4 and modified to contain a non-native disulfide bond.

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79. (once amended) A polypeptide having at least 60% identity to the polypeptide of Seq. ID No. 6 and modified to contain a non-native disulfide bond.

80. (once amended) The polypeptide of claim 79, having at least 70% sequence identity to the polypeptide of Seq. ID No. 6.

81. (once amended) A polypeptide having at least 60% sequence identity to the polypeptide of Seq. ID No. 8 and modified to contain a non-native disulfide bond.

82. (once amended) The polypeptide of claim 81, having at least 70% sequence identity to the polypeptide of Seq. ID No. 8.

83. (once amended) A polypeptide having at least 60% sequence identity to the polypeptide of Seq. ID No. 10 and modified to comprise a non-native disulfide bond.

84. (once amended) The polypeptide of claim 83, having at least 70% sequence identity to the polypeptide of Seq. ID No. 10.